Cells on chips

Jamil El-Ali[1], Peter K. Sorger[2] & Klavs F. Jensen[1]

Microsystems create new opportunities for the spatial and temporal control of cell growth and stimuli by combining surfaces that mimic complex biochemistries and geometries of the extracellular matrix with microfluidic channels that regulate transport of fluids and soluble factors. Further integration with bioanalytic microsystems results in multifunctional platforms for basic biological insights into cells and tissues, as well as for cell-based sensors with biochemical, biomedical and environmental functions. Highly integrated microdevices show great promise for basic biomedical and pharmaceutical research, and robust and portable point-of-care devices could be used in clinical settings, in both the developed and the developing world.

In their normal environment, cells are subject to multiple cues that vary in time and space, including gradients of cytokines and secreted proteins from neighbouring cells, biochemical and mechanical interactions with the extracellular matrix (ECM), and direct cell–cell contacts (Box 1). Microfabricated systems can present cells with these cues in a controllable and reproducible fashion that cannot easily be achieved by standard tissue culture, and can be used to link cell culture with integrated analytical devices that can probe the biochemical processes that govern cell behaviour. Some cell-based microsystems simply represent miniaturized versions of conventional laboratory techniques, whereas others exploit the advantages of small length scales and low Reynolds numbers[1], such as favourable scaling of electrical fields and the ability to create well-controlled laminar flows. In this Review, we discuss the application of microtechnology to cell biology and describe methods for cell culture, regulation of extracellular cues, cell fractionations and biochemical analysis on a micrometre scale (Fig. 1). Emphasis is placed on microsystems aimed at gaining biological insight, as well as on efforts to realize increasing cell-handling integration and biochemical analysis levels on chips.

We believe these devices will become increasingly implemented in applied and basic biomedical research, mainly because soft lithography[2] has put microfluidics within the reach of biology-focused academic laboratories. Elastomeric materials used in soft lithography, typically poly(dimethylsiloxane) (PDMS), are relatively easy to fabricate, and are compatible with most biological assays. Devices that are based on microfabrication of silicon and glass require access to advanced cleanroom facilities similar to those used for microelectronics. This typically involves higher cost, but has unique advantages for specialized applications, such as electrophoresis in glass devices.

Much cell-based microsystem research takes place under a 'lab-on-a-chip' or 'micro-total-analysis-system' (μTAS) framework that seeks to create microsystems incorporating several steps of an assay into a single system[3–5]. Integrated microfluidic devices perform rapid and reproducible measurements on small sample volumes while eliminating the need for labour-intensive and potentially error-prone laboratory manipulations. Thus, microfluidics allows experiments to be carried out that cannot be performed simply by miniaturizing and mechanizing conventional laboratory procedures using robotics and microplates. For example, in cell-based studies, the transition from 384- to 1,536-well plates is proving challenging, largely because edge effects and uncontrolled evaporation from very small wells result in poorly defined culture conditions. Conventional handling of very small fluidic volumes is difficult, and subject to both variability and high fixed losses. The fabrication of many copies of an analytic device, small reagent volumes, and diminished labour associated with use of automated microfabricated devices

Box 1 | Cell physiology, phenotype and fate are regulated by cell-autonomous processes and extracellular signalling molecules

Soluble signalling molecules include hormones, cytokines and growth factors produced by local or distant cells (giving rise to paracrine and endocrine signals, respectively), and even by the receiving cell itself (autocrine signals). Insoluble signalling molecules include components of the ECM and membrane-bound proteins on neighbouring cells. Cells sense most extracellular signals (including proteins, peptides and carbohydrates) via transmembrane receptors that activate complex biochemical cascades of kinases, proteases, adaptor proteins, transcription factors and so on, which together act to regulate cell physiology. At the same time, cells alter their surrounding environment by making or destroying ECM or soluble factors and by exerting mechanical force[99,100].

In animals, cells typically reside in environments with very specific three-dimensional (3D) features. Cells are sensitive to the presence of neighbouring cells of similar or different type and often make long-lasting mechanical and biochemical connections to them. In columnar epithelia, for example, identical cells lined up side by side assemble junctions with neighbours to form continuous impermeable sheets. These sheets are polarized such that cells interact with their surroundings in very different ways on the lumenal and basolateral surfaces. In addition, epithelia usually establish a close relationship with specific types of immune cell. In these epithelia, both homotypic and heterotypic cell–cell interactions are essential to maintain cell and tissue function.

Most *in vitro* experiments with adherent human cells are performed in two-dimensional (2D) cultures in which cells are plated onto plastic surfaces treated to stimulate cell binding. Depending on their type, cells either grow directly on the plastic, secrete ECM components that coat the plastic to facilitate cell adhesion, or require pre-coating of the plastic with ECM. Standard 2D culture conditions are poor mimics of the cellular environment in an animal: soluble growth factors are present at abnormally high concentrations, 3D cues are largely absent, oxygen tension is too high and cell–cell interactions are rarely organized. Attempts have been made to overcome these problems using organ culture and various laboratory-scale bioreactors, but microsystems provide a much more effective means of controlling cell environment *in vitro*. Particularly promising are various artificial organ systems in which multiple cell types are grown together under conditions that mimic normal 3D environmental and circulatory cues.

[1]Department of Chemical Engineering, and [2]Department of Biology and Biological Engineering, Center for Cell Decision Processes, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.

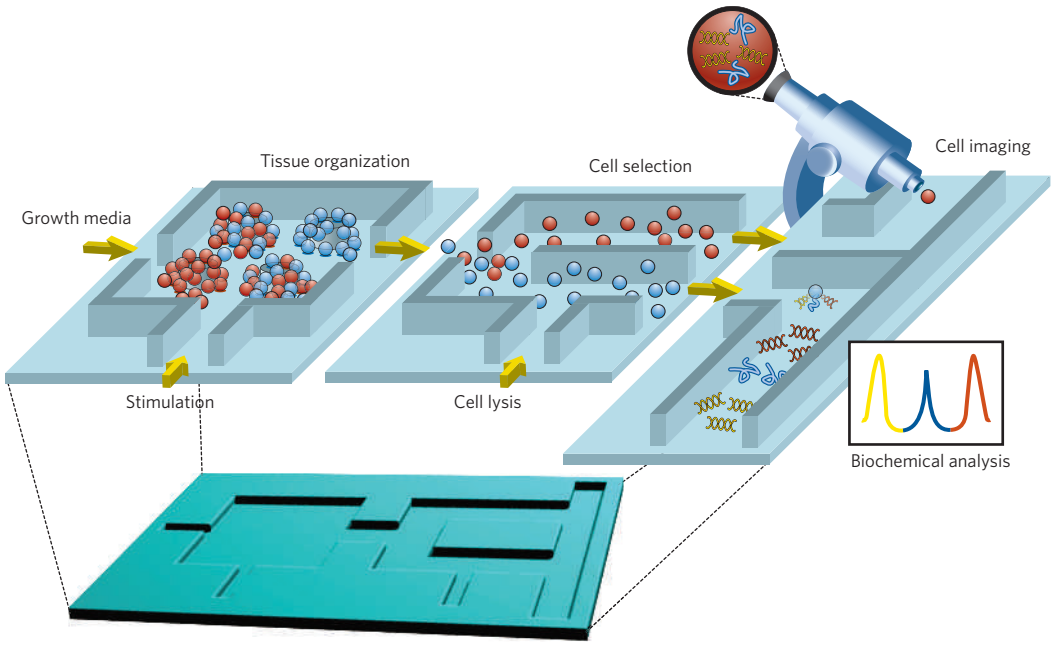

Figure 1 | Tissue organization, culture and analysis in microsystems. Advanced tissue organization and culture can be performed in microsystems by integrating homogeneous and heterogeneous cell ensembles, 3D scaffolds to guide cell growth, and microfluidic systems for transport of nutrients and other soluble factors. Soluble factors — for example, cytokines for cell stimulation — can be presented to the cells in precisely defined spatial and temporal patterns using integrated microfluidic systems. Microsystems technology can also fractionate heterogeneous cell populations into homogeneous populations, including single-cell selection, so different cell types can be analysed separately. Microsystems can incorporate numerous techniques for the analysis of the biochemical reactions in cells, including image-based analysis and techniques for gene and protein analysis of cell lysates. This makes microtechnology an excellent tool in cell-based applications and in the fundamental study of cell biology. As indicated by the yellow arrows, the different microfluidic components can be connected with each other to form an integrated system, realizing multiple functionalities on a single chip. However, this integration is challenging with respect to fluidic and sample matching between the different components, not least because of the difficulty in simultaneously packaging fluidic, optical, electronic and biological components into a single system.

should make them highly cost effective. Moreover, the small footprint and low power consumption of integrated systems creates opportunities for portable, point-of-care devices that can perform analyses hitherto possible only in the research or clinical laboratory. Devices such as these with sophisticated diagnostic capabilities are likely to become important in the personalization of medical care.

Many of the promises of μTAS have yet to be realized: integration and packaging of several functionalities into a single system is proving to be a complex task (Fig. 1), and many cell-based microsystems available today are still in the proof-of-concept phase. Typical unit operations (for example, growth, treatment, selection, lysis, separation and analysis) have been demonstrated (Fig. 2), but robust approaches to fabrication, integration and packaging (such as communication with the macroenvironment) remain major areas of research.

Microfabricated cell cultures

Culturing cells *in vitro* is one of the cornerstones of modern biology. Nevertheless, even for intensively studied tissues, many of the factors that induce or stabilize differentiated phenotypes are poorly understood and difficult to mimic *in vitro*[6]. One approach to increase control over cell–cell and soluble cues typical of *in vivo* cell environments is to combine microfabrication of 3D ECM structures and microfluidic networks that transport soluble factors such as nutrients and oxygen. Microfluidics has the additional advantage of being capable of creating mechanical strain, through shear, in the physiological range.

Cells and the extracellular matrix

Microfabrication integrating micropatterning techniques with advanced surface chemistry makes it possible to reproducibly engineer cell microenvironment at cellular resolution. A large variety of surface-patterning techniques are available, including standard photolithography liftoff techniques, photoreactive chemistry and, increasingly, techniques based on soft lithography (microcontact printing and fluidic patterning)[7]. Surface patterning of micrometre-sized features allows micrometre-scale control over cell–ECM interactions and can be used to generate ensembles of cells with defined geometry. Lamination, moulding and photo-polymerization techniques all allow fabrication of 3D scaffolds with feature sizes in the lower micrometre range, including microstructured scaffolds made of biodegradable materials[8].

The precise control of the cellular environment that has been made possible by microtechnology provides new opportunities for understanding biochemical and mechanical processes responsible for changes in behaviour such as the effects of cell shape on the anchorage-dependence of cell growth[9,10]. For example, by altering the spacing of a grid of cell-adhesive islands it is possible to control the extent of cell spreading, while keeping the cell–ECM contact area constant[10] (Fig. 3a). Human capillary endothelial cells confined to closely spaced islands undergo apoptosis, whereas cells that can spread freely survive and proliferate normally[10]. Adhesive ECM patches can also be designed so that the locations of focal adhesions (integrin-mediated links between the ECM and actin cytoskeleton) result in the same overall cell shape, but with a different underlying cytoskeletal organization (Fig. 3b). By allowing cells to spread and proliferate on these adhesive patches the orientation of the cell division axis can be controlled[11]. Similar regulation of the division axis by the ECM is likely to be important for tissue morphogenesis and other developmental processes.

The force exerted on the ECM by cells can be measured in several ways. A particularly powerful method involves measuring the deflection of arrays of micrometre-sized vertical elastomer posts (Fig. 3c). When tested with smooth muscle cells, forces acting in the plane of the substrate are in the range of 100 nN, and appear to scale with the area covered by focal adhesions[12]. Compared with conventional methods that rely on substrate distortion, the elastomer-post technique has

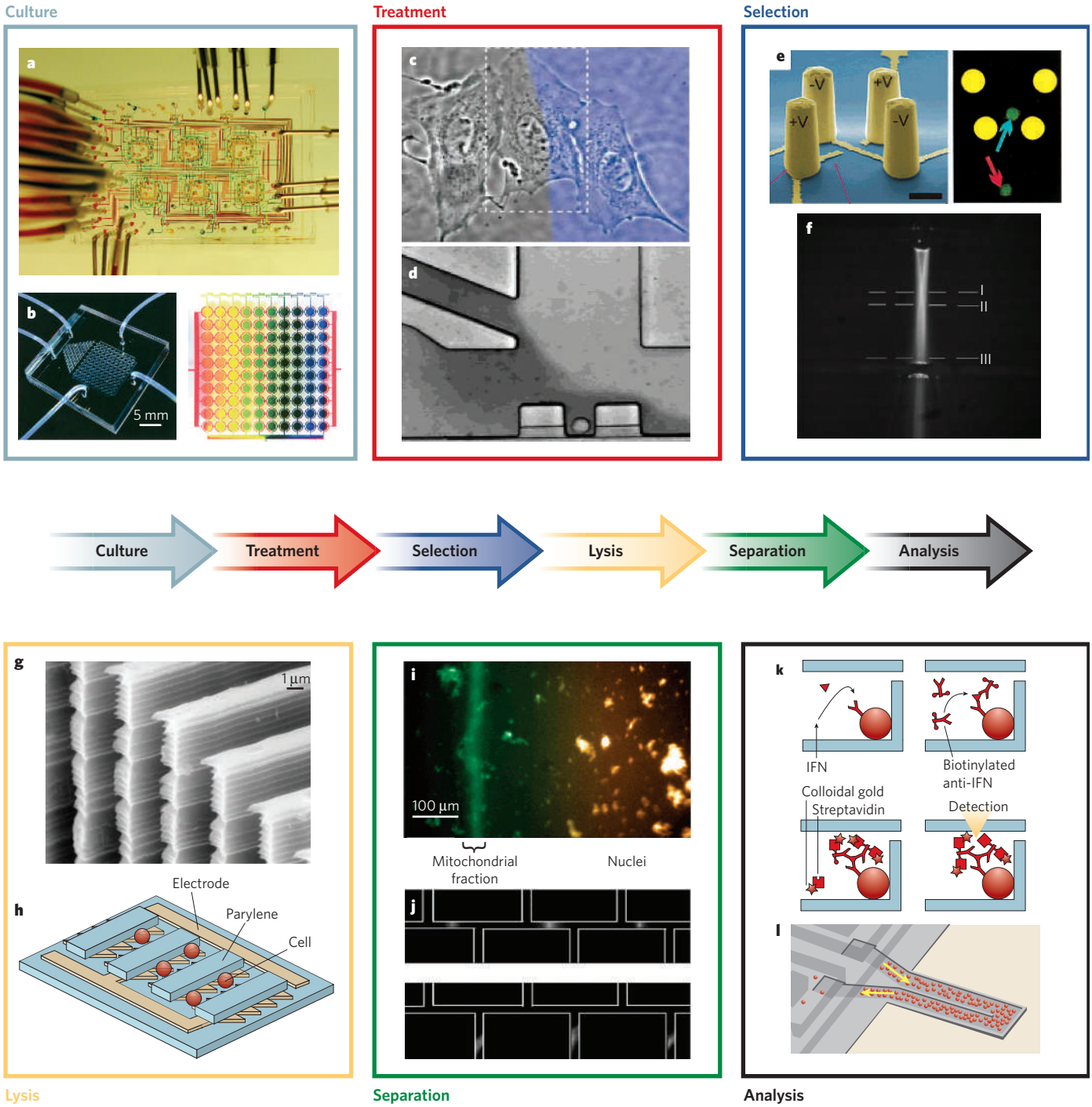

Figure 2 | Microsystems enabling cell-based assays from cell culture to biochemical analysis. A collection of microsystems enabling cell-based assays, covering all the steps from cell culture, through selection and treatment, to biochemical analysis. **a**, Image showing six bioreactors that can operate in parallel on a single chip. Each reactor can be used to monitor the growth of extremely small numbers of cells. (Image reproduced, with permission, from ref. 20.) **b**, Microfluidic cell-culture array with integrated concentration gradient generator (left). Image of concentration gradient across ten columns when loaded with blue and yellow dye. (Image reproduced, with permission, from ref. 33.) **c**, Two different laminar streams exposing two sides of a single cell to different conditions[34]. **d**, Perfusion over a single hydrodynamically trapped cell. Switching of the perfused media can occur in ~100 ms. (Image reproduced, with permission, from ref. 38.) **e**, Single-cell dielectrophoresis (DEP) trap, consisting of four electroplated electrodes (left). Fluorescent image of a trapped cell (indicated by blue arrow; right). The cell has been loaded with calcein through the microfluidic system. (Image reproduced, with permission, from ref. 46.) **f**, Fluorescent image of light path at the detection zone in a micro flow cytometer with integrated waveguides and lenses. (Image reproduced, with permission, from ref. 53.) **g**, Scanning electron micrograph of a mechanical lysis device with sharp knife-like protrusions. (Image reproduced, with permission, from ref. 55.) **h**, Schematic of electrical lysis device with integrated microelectrodes. (Image reproduced, with permission, from ref. 56.) **i**, Isoelectric focusing of cell organelles from whole-cell lysate. The mitochondria focuses in a band at pI between 4 and 5. (Image reproduced, with permission, from ref. 62.) **j**, Two-dimensional separation of four model proteins. Isoelectric focusing (top) followed by SDS gel electrophoresis. (Image reproduced, with permission, from ref. 64.) **k**, Schematic of immunoassay performed using microbeads as solid support in a microfluidic system. (Image adapted, with permission, from ref. 69.) **l**, Schematic of a hollow cantilever-based mass sensor for analyte detection. (Image adapted, with permission, from ref. 74.)

the advantage of greater accuracy and manipulability: the mechanical properties of a surface can be varied by changing post geometries without altering surface chemistry[12].

Liver-cell culture

In vitro culture of liver cells has received particular attention in biotechnology as many drugs fail in clinical studies either because they damage the liver directly or because liver metabolites are toxic[13]. The study of hepatotoxicity would be greatly facilitated by the availability of *in vitro* culture systems that mimic real liver conditions. However, the development of liver-cell cultures as biosensors for drug toxicity faces challenges because of the difficulty in maintaining the differentiated phenotypes.

In the liver, hepatocytes are found in a complex 3D environment in which nutrients, soluble factors and oxygen are transported through blood capillaries and bile canaliculi. Using silicon as a substrate, perfused 3D liver reactors have been fabricated on arrays of 300-μm-wide channels (capillaries) that comprise a scaffold for the ECM[14] (Fig. 3d). Seeding hepatocytes with pre-aggregated multicellular spheroids in the 3D reactor generates cultures that are viable for a long time period (~3 weeks) and that exhibit a stable differentiated phenotype. Cells in 3D liver cultures also have cell–cell contacts, such as tight junctions and desmosomes, that resemble those found in tissues *in vivo*[13,14].

It has been observed that co-culture of hepatocytes with other cell types, including liver epithelial cells and Kupffer cells, prolongs the survival of cultured hepatocytes and helps maintain liver-specific properties such as albumin secretion[15]. Using a micropatterned 2D co-culture system, it has also been shown that liver-specific functions increase with heterotypic cell–cell interactions. Only hepatocytes close to the heterotypic interface maintain their differentiated phenotypes in longer-term culture[6] (Fig. 3e). Relative to conventional co-culture, in which seeding densities of two cell types are varied on a planar surface, micropatterning techniques afford greatly improved control of homo- and heterotypic cell–cell interactions[16]. The ability to culture cells such as liver cells *in vitro* and to demonstrate protein and gene expression levels similar to those found in tissue suggests that microfabricated cultures could have applications in toxicology and could also serve as model systems for *in vitro* analogues of organ tissue.

Bone

Bone loss after menopause, long periods of inactivity or life in a microgravity environment poses a serious medical problem. Bone is a tissue in which shear stress and mechanical loading are important. Mechanical interactions are necessary for maintaining cultured osteoblastic cells in a state suitable for bone engineering. In standard 2D culture, shear stresses as low as 10 μPa enhance differentiation of osteoblasts as measured by alkaline phosphatase activity and fibronectin expression[17]. Microtechnology provides an opportunity to build 3D scaffold and fluidic networks that mimic the natural 3D environment of bone. This includes the use of fluidics to deliver soluble factors to the cells and to impose shear stress at the physiological level. In a 3D network of microstructured channels, alkaline phosphatase activity in osteoblast cells is enhanced threefold under static conditions (corresponding to a structural effect) and 7.5-fold under low flow conditions (representing a combined structural and shear effect) relative to 2D static cultures[18].

Microrganisms

Microsystems also have applications as tools to screen and optimize conditions for yeast and *Escherichia coli* fermentation and growth during bioprocessing. Microfluidic bioreactors have been miniaturized to create nanolitre growth chambers in which extremely small cell populations can be monitored[19,20] (Fig. 2a). The use of small reactor volumes and multiple independent cell populations helps to decrease problems associated with genetic variation and makes it possible to assess many different conditions in parallel. The ability to integrate optical sensors in growth chambers also makes it possible to monitor key process variables such as pH, dissolved oxygen and biomass[21]. Data on these variables could be combined with gene expression analysis and metabolic studies to rapidly prototype and then scale up conditions for industrial bioprocessing[22].

Cell stimulation and selection

The control of cellular microenvironments via microfluidic systems potentially represents a valuable tool for fundamental studies of cell biology. Biological insight into the pathways that control cell phenotype and behaviour can be gained by monitoring cellular responses to controlled perturbations in the extracellular environment. A wide range of microsystems are therefore emerging with the express aim of facilitating the basic study of biochemical pathways, cell-fate decisions and tissue morphogenesis. In the next two sections, we provide examples of some techniques being applied to cell-based assays (Fig. 2). Readers are also referred to more detailed reviews elsewhere[23–29].

Cell stimulation of adherent cells

Controlled perturbation of the cellular environment in time and space for adherent cells in microfluidic devices can be accomplished by controlling flow over the cells. As mentioned above, fluid flow not only transports soluble factors, but also exerts mechanical force through shear[14,18]. The diffusive mixing properties of laminar flow created by microfluidics can also be used to create complex concentration gradients not achievable on a macroscale[30]. These gradients allow several conditions to be probed simultaneously while also mirroring conditions found *in vivo*. For example, repeated combinations of flow-stream lamination and splitting can create complex concentration gradients that promote cell chemotaxis[30] — the migration of cells in response to a stimulus. Some bias in the cell migration due to shear is observed in systems with high flow rates[31]. Linear gradients of external factors can also be created in static (convection-free) microfluidic systems without perturbing the existing distribution of secreted molecules, thereby preserving autocrine and paracrine signalling[32]. For cell culture applications, gradient generation permits many growth conditions to be analysed in a combinatorial fashion[33] (Fig. 2b).

In systems with fast flow or large molecules (small diffusion coefficients), diffusion is often too slow for any appreciable mixing between fluidic streams. Although slow diffusion poses a complication when mixing is desired, slow diffusive mixing creates opportunities for varying the liquid-phase environment over distances comparable to the size of cells. For example, laminar flow has been used to expose two halves of an endothelial cell to different mitochondrial dyes, making it possible to observe the movement of organelles from one side of a cell to the other[34] (Fig. 2c). A similar approach, based on temperature steps rather than a chemical gradient, has recently been used to study the effects of temperature perturbations on embryonic development in *Drosophila*[35].

Cell stimulation in suspension

Cells in suspension are usually transported with flow. However, cells can be physically retained in the devices by filters or traps. By combining concentration gradients and flow of cells with hydrodynamic traps that retain cells in a fixed position, microfluidics has been used to monitor ATP-dependent calcium uptake in HL-60 cells[36]. Hydrodynamic traps placed on either side of a narrow microfluidic channel can also be used to capture cells so that well defined cell–cell contacts form[37]. Trapping single cells near a two-channel reagent-delivery system can also enable very rapid (within ~100 ms) fluidic switching between a buffer and a reagent stream at the cell position. This makes it possible to monitor very fast cellular responses, such as calcium flux in ionomycin-stimulated Jurkat cells[38] (Fig. 2d).

If cells are allowed to flow through a device with the bulk liquid, they will spend a characteristic time in each fluidic compartment. Soluble cues can then be applied to cells for varying time periods by mixing in reagents in successive fluidic compartments. For example, by using a flow segmentation technique to enhance mixing, fast transient mitogen-activated protein kinase responses of α-CD3-stimulated Jurkat cells have been monitored with excellent reproducibility and temporal control[39]. Flow segmentation enhances mixing by creating a small recirculation within each segment[40]. Analysis of stress markers shows that segmented microfluidic flow does not trigger significant cell stress responses[39].

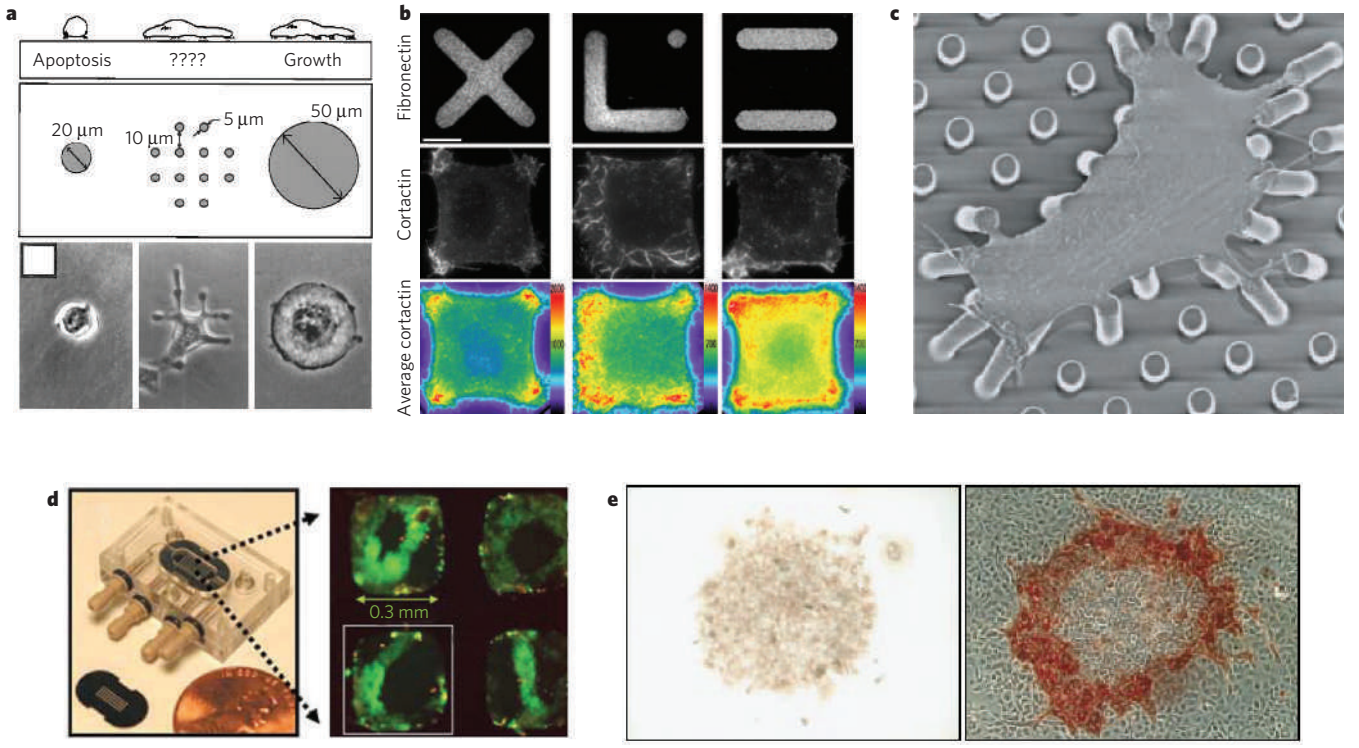

Figure 3 | Substrate patterning and tissue culture. **a**, Diagram of substrate patterns that can be used to control the area cells can spread over without varying the cell–ECM contact area. The corresponding images show that if cells are confined to a small area, they undergo apoptosis, whereas if they are allowed to spread over a larger area while keeping the same cell–ECM contact area, they remain viable. (Image reproduced, with permission, from ref. 10.) **b**, The membrane ruffles, revealed by the cortactin marker, are preferentially located where the cell membrane attaches to the fibronectin in the ECM[11]. **c**, Cell cultured on an array of compliant micro-posts. The direction and magnitude of the deflection of the posts is a measure of the local force field. (Image reproduced, with permission, from ref. 12.) **d**, Assembled liver-cell microfluidic system with four ports for fluidic access. A viability stain shows that most cells in the scaffold are viable (green) and there are only few non-viable cells (red). (Image reproduced, with permission, from ref. 13.) **e**, Immunostaining of intracellular albumin in micropatterned hepatocyte cultures. Cells in the homoculture (left) have lost albumin after 6 days of culture. In the heteroculture (right), hepatocyte cells near the heterotypic interface retain albumin content at day 6, whereas cells away from this interface lose albumin content. (Image reproduced, with permission, from ref. 6.)

Cell sorting

Fluidic transport with selective trapping or diversion of suspended cells allows cell sorting to be integrated into microfluidic systems — a powerful capability when combined with the methods for cell stimulation. The ability to isolate homogeneous and concentrated cell populations from heterogeneous cell mixtures can also be important for obtaining accurate information about the underlying biochemistry of specific cell types in a mixture. Microtechnology makes it possible to isolate a few cells (or even single cells) from a large population of cells on the basis of physical and chemical properties such as electrical characteristics or fluorescent markers, ultimately allowing heterogeneities within seemingly homogeneous cell populations to be exposed[41].

The ease of integrating electrodes along with the favourable scaling of electrical fields in microsystems creates opportunities for exploiting dielectrophoresis (DEP) to move, separate and position individual cells[42]. The DEP force arises from induced dipoles in cells exposed to a non-uniform electrical field. DEP depends on the intrinsic electrical properties of a cell, such as membrane capacitance and conductance, both of which change with cell type and even with cell activation[43]. For example, by using DEP and microfluidics, MDA231 cancer cells have been separated from dilute blood by selective capture onto microelectrodes[44]. Tagging cells with marker particles with different dielectric properties has allowed DEP sorting for rare cells at rates up to 10,000 cells s^{-1} and with enrichment factors of more than 200 (ref. 45). With proper electrode design, DEP can be used to capture and manipulate single cells, and stimuli can be introduced via the fluidic system, for example, to monitor the kinetics of fluorescent dye (calcein) uptake in HL-60 cells[46] (Fig. 2e). Each trap can then be made electrically addressable for selective capture and release of cells for further analysis. The importance of separating cell populations before biochemical analysis is exemplified by the DEP-based separation of U937 cells and peripheral blood mononuclear cells (PBMCs) into two homogeneous populations. Following cell stimulation, increases in the expression of cytokine genes can be detected in sorted populations of U937 cells, but the effect is almost completely masked in mixed-cell populations[47].

Fluorescently activated cell sorters (FACS) have also been miniaturized using integrated pneumatically activated pumps and valves that divert cells into a collection chamber on the basis of their fluorescent properties[48]. Depending on cell concentrations and purity, cells can be sorted at rates of up to ~40 cells s^{-1} with enrichment factors of ~90 and recovery yields of between 16 and 50%. Using optical forces instead of mechanical valves to switch the direction of cells permits a slightly higher throughput of ~100 cells s^{-1}, with recovery yields above 80% and enrichment factors of up to ~70 (ref. 49).

Cells can also be separated on the basis of the affinity of cell-surface receptors for proteins immobilized on the surfaces of the microfluidic channel[50]. Transient adhesion between cells and appropriate surface ligands retard cell movement through the channel. This creates a chromatographic separation between two cell types on the basis of differences in their retardation. For example, by using selectin as an adhesion molecule, HL-60 cells can be separated from U937 cells, albeit with low resolution[50]. A similar affinity-based capture technique has been used to capture T cells in microwells using anti-CD5 as a surface ligand[51].

Although many microfluidic sorting systems achieve high levels of integration with electrodes, valves and even pumps, most microsystems rely on bulk optical elements such as lenses and microscopes external to the microdevice for optical control and detection. It is not uncommon to see a microchamber coupled to lenses and electronics that occupy an entire optical table. Miniaturization of free-space optical elements is not

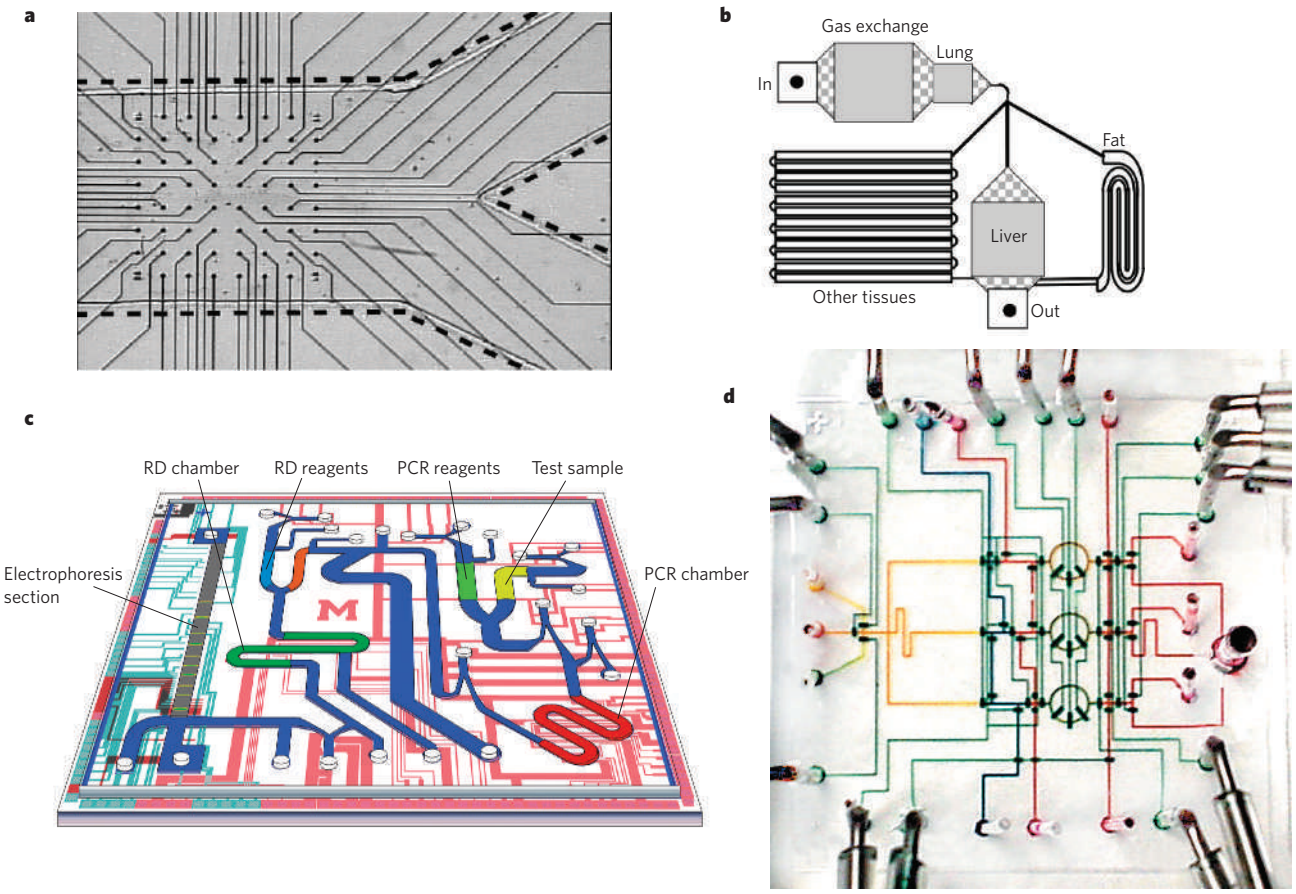

Figure 4 | Integrated cell analysis systems. **a**, Microelectrode array for recording neuronal activity integrated with a microfluidic channel. (Image reproduced, with permission, from ref. 81.) **b**, Cell culture analogue with four different interconnected tissue compartments. The lung and liver chambers contain cells, whereas the 'other tissues' and fat compartments have no cells but mimic the fluid residence-time distribution in tissues of rapid and slow perfusion, respectively. (Image adapted, with permission, from ref. 89.) **c**, Schematic of an integrated system for genetic analysis. The system can perform two independent serial biochemical reactions — polymerase chain reaction (PCR) amplification of a sample followed by restriction endonuclease digestion (RD). Analysis is performed by electrophoretic separation and fluorescent detection. (Image reproduced, with permission, from ref. 93.) **d**, DNA extraction and purification chip, with advanced integrated fluidic handling of cell samples as well as the necessary buffers and reagents[96].

easy, but efforts are underway to integrate optics with microsystems[52]. Recently, a microchip-based flow cytometer with integrated polymer waveguides and lenses was described[53] (Fig. 2f).

Most integrated microflow cytometers have yet to match conventional systems in performance (>10,000 cells s^{-1}), but they have smaller footprints, are of lower cost, and create opportunities for in-line integration with other analytic devices. Furthermore, with the laminar-flow conditions found in microsystems, the sorting of viable cells does not appear to perturb cell physiology appreciably[47,49].

Biochemical analysis of cell lysates

A significant research effort is devoted to the development of integrated tools for microscale biochemical analysis. Quantitative analysis of complex biochemical mixtures, such as cell lysates, remains challenging, and with many devices success has only been achieved with low-complexity samples. Nonetheless, almost every analytical tool available in a conventional biology lab has an equivalent microfabricated counterpart, and many of these have been nicely summarized in reviews[23–25,27]. Protein analysis is generally more difficult than analysis of nucleic acids, because the physical and chemical properties of proteins are much more variable than those of either RNA or DNA. Moreover, unlike DNA and RNA, no methods exist for amplifying proteins. Relative abundance of proteins in cell lysates can vary by more than 10^5, making sensitivity and dynamic range critical to any successful assay. The problems of low abundance and high complexity are generally handled in one of two ways: by linking sample preparation steps such as physiochemical separation and concentration before analysis, or by using high selectivity in the analytical system, typically through affinity methods based on antibodies. In addition, it is important to apply surface coatings to limit non-specific surface absorption, which results in significant loss of material.

Cell lysis

The lysis of cells in the laboratory is generally accomplished either chemically, with detergents, or mechanically, by membrane rupture. Microfluidic devices can incorporate either method. Chemical lysis with Triton X-100 (ref. 39) and denaturation with sodium dodecyl sulphate (SDS)[54] have both proved effective on a microscale. The main advantage of chemical lysis is that subsequent assays can be performed in buffers previously optimized for conventional biological studies. Mechanical cell lysis is an alternative method if detergents interfere with downstream analysis. For example, microscale shear lysis similar to conventional macroscopic techniques can be achieved by forcing HL-60 cells through nanoscale barbs[55] (Fig. 2g). Although lysis — as monitored by release of an intracellular dye — is almost complete, aggregation of the cellular debris results in such inefficient protein extraction that only ~5% of total protein is accessible for subsequent analysis. The use of electroporation as an alternative to mechanical lysis has been motivated by its ability to achieve high local fields using integrated microelectrodes[56,57] (Fig. 2h). Electrical lysis can be rapid, with disruption times as low as 33 ms — about eight times faster than lysis by SDS[58]. By carefully controlling the strength of the electrical field, microfabricated electroporation devices can also reversibly destabilize the cell membrane for gene transfection applications[59].

Sample preparation

Protein and DNA fractionation has been achieved using microfabricated sieving systems with nanometre-sized filters[60,61]. However, microsystems that rely on electrokinetic separation techniques, such as capillary electrophoresis (CE), gel electrophoresis, electrochromatography or isoelectric focusing (IEF)[23,24,27] are more commonly used. These microsystems can typically achieve faster separation with smaller volumes than their conventional counterparts. The use of electrokinetic techniques also extends to the separation of organelles. Mitochondria from HeLa cells undergoing apoptosis have been fractionated from each other and from nuclei using IEF[62] (Fig. 2i). Increased resolution in electrokinetic protein separation on the microscale can be achieved by using a 2D separations system similar to those used on the laboratory scale — for example, a combination of electrochromatography with CE[63], or IEF with gel electrophoresis[64] (Fig. 2j). Electrokinetic techniques can also be used to concentrate proteins and peptides significantly in a sample[65].

The ability to amplify DNA and RNA using PCR and reverse transcription PCR (RT-PCR) is a very powerful tool that allows sensitive detection and quantification of nucleic acids. Microdevices for PCR amplification integrate electrodes for heating and temperature sensing used to control the thermal reactions of the PCR cycle. The low thermal mass of microsystems results in very fast temperature cycling, with heating and cooling rates in excess of 35 $^{\circ}C\ s^{-1}$ (ref. 66). DNA amplification can also be directly integrated with electrophoretic separation techniques for complete sample pretreatment systems[67].

Analytical techniques

A large number of techniques exist for the quantification of cell lysates. A major advantage of microsystems containing integrated electrophoretic separation technology is that detection and quantification are possible by absorption techniques or by using relatively simple fluorescent markers. After separation, the sample simply passes by an optical detector. Microsystems with electrophoretic separation are also increasingly being coupled to mass spectrometers for protein identification and the analysis of post-translational modifications[23,24,68].

One quantitative method for protein analysis and detection that is widely used in microsystems and does not require prior fractionation of even complex biological samples is antibody capture. Antibody-based techniques rely on the high selectivity and affinity of antibody–antigen binding to achieve specificity in analysis[24,27]. Integrated microfluidic systems can coat either solid supports[69] (Fig. 2k) or channel surfaces[70] with capture antibodies, and subsequently introduce the analyte and secondary detection antibodies, if necessary. Similar microsystems have been developed for DNA hybridization, with some devices able to carry out a hybridization assay on 1 μl of sample in less than 10 min (ref. 71).

Label-free detection represents a potentially powerful alternative to fluorescent and luminescent detection. Generally applicable methods include detection of changes in mass and electrical properties due to binding on antibody-functionalized sensor surfaces. Field-effect sensors have been realized with carbon nanotubes[72] and silicon nanowires[73] as the active sensing elements. Recently described cantilever technologies look promising as mass-based sensor techniques[74,75] (Fig. 2l). The extensive area available for affinity capture and the sensitivity of these systems should allow them to detect femtomoles of protein.

The ability of analytical microfluidic systems to reproducibly handle small samples and high throughput, combined with the integration of sample preparation and separation steps, has resulted in these systems finding considerable commercial applications in the fields of genomics and proteomics[76].

Applications

Cell-based microdevices, including biosensors, are increasingly being used in drug discovery, genetic analysis and single-cell analysis. This section describes some recent examples, although many others can be found in the literature, and new devices and applications continuously emerge.

Biosensors

Cell-based biosensors monitor physiological changes in reporter cells exposed to biological or industrial samples containing pathogens, pollutants, biomolecules or drugs[77,78]. The readout can be optical (for example, fluorescent, luminescent or colorimetric) or electrical (for instance, measuring changes in impedance or electrical potential)[79]. Some biosensors detect simple phenotypic responses, such as life versus death.

The chemical-dependent electrophysiological activity of certain cell types, such as neurons and cardiac cells, has spurred their use in chip-based biosensors. Changes in electrical activity can be monitored by planar microelectrode arrays[80], which are easily integrated into microfluidic devices and can be made with large numbers of measurement points per device[81] (Fig. 4a). ECM surface patterning makes it possible to place neurons at precise points on the electrode arrays, and patterning can also be used to modulate the activity of the assembled neural networks *in vitro*[82]. Microfluidics can not only deliver soluble factors, but also present topographical cues that help to control neuronal connectivity[83]. A portable, highly integrated cell-based biosensor system for the analysis of biochemical agents has been realized by integrating a complementary metal oxide semiconductor (CMOS) chip as digital interface with recording electrodes as well as with a temperature control system that includes heater electrodes to sustain the environmental requirements of the cells in the microfluidic culture chamber[84]. The system has been tested with a 5-μM solution of a calcium channel blocker (nifedipine). Challenges still remain in using living cells as sensors, because variables such as cell density and cell interaction can significantly affect the sensor properties.

Drug screening

High-throughput measurement of ion-channel activity by patch clamping is of considerable interest in drug discovery as a tool to characterize therapeutic molecules. Microsystems that combine high throughput with small reagent volumes have led to commercial microscale patch-clamp devices[85]. In these devices, ion-channel recording is typically achieved by placing cells on a micrometre-sized aperture in a membrane that separates two electrodes[86,87]. By guiding cells onto apertures using microfluidic paths, it is possible to reduce the otherwise labour-intensive micromanipulations needed to locate cells at recording sites and to present the cell with successive stimuli[88]. Obtaining the high-electrical-resistance seals necessary for high quality ion-channel recording ($\sim 10^9\ \Omega$) is technically challenging on both a macro- and a microscale, and microsystems have been more successful in meeting the throughput challenge.

One challenge in drug and toxicology screening is recreating the cell–cell interactions found in living organisms. The toxic effect of many drugs in a target tissue often depends on the metabolic activity of another tissue — in particular the liver. In such situations, tests of a drug on two tissues in isolation would not necessarily reveal any toxicity. This limitation has been addressed by constructing a microsystem using interconnected channels and chambers, each of which contains a different cell type mimicking the activity of a particular tissue[89] (Fig. 4b). The interconnected compartments for the liver, lungs and fat cells are designed to capture physiologically relevant features, such as residence times, of the circulation and interchange of metabolites in the body. However, challenges remain in maintaining differentiated phenotypes and matching fluidic conditions in different compartments[89].

Stem cells

The promise of stem cells for cell-based therapies in human disorders and tissue engineering has resulted in a growing interest in applying microtechnology to stem-cell culture. The controlled microenvironment of microfluidic platforms can be very useful in the study of stem cells[90]. Manipulating the chemical environment of the culture in time and space allows the behaviour of stem cells, such as proliferation and differentiation, to be controlled.

A microfluidic stem-cell culture platform with a concentration gradient has been used to study the effect of growth-factor concentration on human neuronal-stem-cell behaviour[91]. The observed proliferation rate in the device was proportional to growth-factor concentration,

whereas differentiation (to astrocytes) was inversely proportional. In these studies, flow in the device minimized autocrine and paracrine signalling. However, it is also possible to set up a linear concentration gradient in a static microfluidic system, preserving autocrine and paracrine signals[32]. Recently, a microfluidic device for stem-cell culture with both logarithmic varying perfusion rates and concentration gradients has been developed, making it possible to explore a wide range of biological conditions (including effect of shear) simultaneously[92]. Future integration of advanced culturing techniques using heterotypic culture and 3D cues is likely to further increase the value of microdevices for stem-cell research.

Genetic analysis

The most developed analytical microsystems so far are those that measure DNA and RNA. These devices often rely on PCR and similar techniques for sample amplification, and include hybridization arrays, real-time probes or electrophoretic sizing for analysis. Pathogen and disease detection can benefit greatly from fast and cheap field-capable devices similar to the one recently developed for the detection of influenza[93] (Fig. 4c). This device integrates valves for precise fluidic handling, temperature control with integrated heaters for DNA amplification by PCR, and electrophoretic separation after restriction endonuclease digestion. The current cost of the device is estimated at US$7 per chip, which could potentially drop below $1 with further scaling down of device dimensions. A similar highly integrated but portable device is capable of bacterial pathogen detection after PCR amplification and electrophoretic separation in less than 10 min, with detection limit as low as 2–3 bacterial cells[94]. Thermal cycling, sample purification and capillary electrophoresis have also been integrated in a device for nanolitre-scale DNA sequencing, allowing more than 500 continuous bases to be sequenced with 99% accuracy[95].

Single-cell analysis

In both conventional studies and microsystems, the analysis of single cells has typically been performed using image-based techniques and intracellular fluorescent probes (such as those that measure calcium flux[38]). However, the ability of integrated microfluidics to accurately manipulate, handle and analyse very small volumes has opened up new opportunities for analysis of intracellular constituents. A microfluidic device with integrated pneumatic valves capable of isolating single cells and then lysing them using a chemical lysis buffer has been shown to be capable of extracting and recovering messenger RNA from a single cell[96] (Fig. 4d). A similar device that also integrates electrophoretic separation can analyse amino acids from the lysed contents of a single cell[97]. Single-cell analysis by electrophoretic separation but with electrokinetic flow-driven cell loading, docking and lysis have also been demonstrated[98].

Outlook

Microfabricated devices have been developed to facilitate both applied and basic research into the biology of cells and tissues. However, many devices have so far only been tested with simple, low complexity samples, and examples of multi-step integration are only now emerging. In many cases in which actual biological specimens have been examined, it has been necessary to fractionate or otherwise process samples before introduction into the microsystem, although nucleic-acid analysis is one exception. Analysis of proteins in clinical samples, such as blood serum or whole-cell lysates, presents challenges. The realization of effective devices for pretreatment or fractionation of complex samples therefore remains a challenge to the practical application of integrated micro-analysis systems in protein chemistry. Integration and automation are important goals that also remain considerable hurdles. The rationales for integration include greater accuracy and reproducibility, smaller sample sizes, and higher throughput. Rather than monitoring only simple phenotypic changes, future integrated systems should be able to gather precise biochemical and mechanistic data from cells and tissues. A fully integrated liver toxicology chip, for example, might include 3D microculture that sustains the differentiated phenotypes of multiple cell types, including hepatocytes, and fluidics to refresh the culture medium and apply biological cues or small molecules. On-line cell separators would facilitate the selection of specific cell populations that could then be delivered to a lysis chamber and, subsequently, to multiplexed on-line sandwich immunoassays using integrated optics or label-free detection. Such systems would require much less material than today's laboratory-scale methods, a huge advantage with primary cells and patient tissues.

The growing emphasis in molecular biology on single-cell analysis derives from increasing appreciation of phenotypic heterogeneity among cells in a population and of the scientific insight that derives from accurately assaying this heterogeneity. Physicochemical modelling of biological processes also demands single-cell data, or at least information about the distributions of key parameters. However, notable challenges remain in the detection of low-abundance proteins, which tend to adhere nonspecifically to surfaces (which are larger per unit volume in many microsystems than in laboratory-scale devices). Trade-offs are likely to exist between measuring more variables and using fewer cells. In our opinion, excessive emphasis on single-cell analysis, rather than on the use of microtechnology to link complex heterogeneous cultures, controlled perturbations and cell fractionation, is unwarranted (Fig. 1).

Although significant challenges face routine applications of 'cells on chips', tremendous advances have been realized over the past decade, and a future in which chips effectively compete with laboratory-scale technologies in the analysis of complex biological phenomena is clearly in sight. Highly integrated microdevices will find application in basic biomedical and pharmaceutical research, whereas robust and portable point-of-care devices will be used in clinical settings. ■

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